

fork structure during DNA replication. In order to gain insights of how HMG2 interacts of DNA forks, we performed single-DNA manipulation studies of the binding of HMG2 to DNA under various DNA structural and topological constraints such as DNA fork with three DNA arms, supercoiled DNA, DNA hairpins, and ssDNA. Ours results show that HMG2 preferentially binds to supercoiled and forked DNA comparing to torsion unconstrained DNA and it has the least binding affinity to single-stranded template. The AT-hook DNA-binding domains in HMG2 are critical form binding - removal of two of the three AT-hooks completely abolishes the binding. In conclusion, our studies show that HMG2 proteins recognize structure rather than specific nucleotide sequences for binding via their unique AT-hook binding domains.

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Single-Molecule Analysis of Transcription-Coupled Repair

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Transcription-coupled repair (TCR), a subpathway of nucleotide excision repair (NER), has been known to lead to more efficient repair than the global NER repair (GGR). Here we use magnetic trapping of single DNA molecules to study the interactions of TCR proteins with stalled RNA polymerase (RNAP). Single RNAP molecules are stalled on a DNA transcript using either a CTP-less cassette or a thymine-thymine dimer located on the transcribed strand. Stalled RNAP is displaced by the Mfd translocase, and a long-lived intermediate is formed. We characterize the interaction between UvrA/UvrB proteins and the long-lived intermediate formed upon Mfd displacement of RNAP. This interaction leads to formation of a pre-incision complex that is catalytically competent for DNA incision, and the activity of this complex is tested for in the magnetic trap upon addition of UvrC.

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Investigation of the Tus-ter Blocking Efficacy during the Chromosome Replication of Live Escherichia Coli Cells

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In *E. coli*, a circular chromosome is replicated in a bi-directional manner by two replisome complexes that assemble at a single origin of replication (*oriC*). The two replication forks are thought to terminate in the termination region, which is flanked by 10 *ter* sites. Each of these sites is bound by a Termination utilization substance (Tus) protein, thereby forming Tus-*ter* complexes. *In vitro* studies have shown that the replication forks are blocked when they approach a Tus-*ter* complex from the non-permissive side, but not from the permissive side. However, the blocking efficacy of the Tus-*ter* complex on a replication fork approaching from the non-permissive side, and the subsequent dynamics, have not been examined in live cells. To shed light on these processes *in vivo*, we utilize quantitative fluorescence microscopy combined with microfluidics to study four different *E. coli* strains that possess either a normal *oriC* or only an ectopic copy (*oriZ*) (inserted 344 kb in the *E. coli* genetic map along the chromosome), and have either wildtype Tus present or knocked-out (*Δtus*). In the *oriZ* strain, the clockwise replication fork (labelled via its sliding clamps) encounters the Tus-*ter* complex (via its non-permissive face) earlier than the counter-clockwise fork does. On tracking the progression of the replication forks in *oriZ* strain, we find that the presence of the Tus-*ter* complex reduces the rate of replication. By monitoring the fluorescence intensity of the fork as well as the duplication of a chromosomal locus, we can determine that a replication fork can nonetheless bypass the non-permissive side of a Tus-*ter* complex. The *oriZ-Δtus* strain exhibits replisome dynamics similar to that of *oriC* strain, whereas the chromosome dynamics differ substantially where the *oriZ* locus moves mostly towards cell-pole.

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Extremophile DNA Photolyases: DNA Repair under Extreme Conditions

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Adaptation to extreme environments is a seminal characteristic of life on Earth. Nowhere is this property more strongly evident than in single-celled organisms. Bacteria, archaea, and eukaryotes all have representatives that thrive in cold or hot environments. Many of these extremophile enzymes share significant amino acid sequence homology in spite of very different optimal growth temperatures (TG). Based on a simple amino acid sequence analysis it is unclear what adaptive changes are required to tailor homologous enzymes to function in extreme environments.

A case in point is DNA photolyase (PL), a monomeric flavoprotein that binds UV-damaged DNA and repairs it by blue light activated picosecond electron

transfer from a conserved flavin adenine dinucleotide cofactor (FADH) to the tightly bound cyclobutylpyrimidine dimer DNA lesion. These enzymes also include a second light-harvesting cofactor. Here we present a comparative analysis of three recombinant CPD photolyases, hyperthermophilic *Sulfolobus solfataricus* (TG=353K, rSsPL), mesophilic *E. coli* (TG=310K, rEcPL), and psychrophilic *Colwellia psychrerythraea* (TG=281K, rCpPL).

All PLs utilize FADH bound in a highly conserved site for repair. In addition, each PL demonstrates different properties for its 2nd cofactor. We used a variety of biochemical, physical, and molecular biological tools to compare these extremophile proteins with regard to repair yield, cofactor reduction potential and excited state properties. The difference in the stability of the redox state of the purified protein suggests different structural adaptations of each PL to their respective thermal environments. Denaturation studies reveal that rSsPL has a very stable structure, whereas rCpPL is extremely sensitive to its thermal and aqueous environments. A comparison of cofactor absorption and emission spectra reveal significant differences in how the flavin cofactor is bound in the binding pocket, in spite of significant amino acid sequence homology for this cavity across all proteins.

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Structure and Nano-Mechanics of DNA during the Initial Stages of Methyl-Directed Mismatch Repair

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DNA undergoing replication is a mechanically complex system on which mismatch repair machinery must not only identify errors on a concurrently replicating / supercoiling DNA molecule but also signal the information along the crowded DNA molecule to excision / re-synthesis elements. In the methyl-directed (dam/mutH-dependent) mismatch repair pathway in *E. coli*, if errors in newly-replicated DNA are detected by MutS, MutH will nick transiently hemi-methylated d(GATC) sites on the daughter strand in response to activating signals from MutS and MutL to initiate repair. The nearest d(GATC) may be over 1000 base-pairs away from the error, and the mechanics of how MutH-activating signals are transmitted along DNA remain poorly understood—most models focus on efforts by MutS/MutL complexes to contact latent MutH waiting on the DNA. Using a suite of single-molecule tools (atomic force microscopy, single molecule force spectroscopy, and tethered particle motion), we directly investigate the structure, forces, and nano-mechanical behavior of individual DNA undergoing the initial stages of repair. To understand how d(GATC) sites are identified after a replication error is found, we observe and biophysically characterize the looping of individual DNA molecules by MutS and the structure of MutS/MutL/MutH complexes on DNA, and record both the mechanical strength of interactions between complexes of MutS, MutL, and MutH and with DNA and the resulting structure of the DNA molecule. Potential application and extension of these biophysical techniques to MutH-independent and mammalian mismatch repair systems will also be discussed.

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Direct Visualization of DNA Replication Conflicts in the Bacterial Cell

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Cell proliferation requires timely and reliable DNA replication. Genetic and biochemical evidence reveals that the replication process is subject to a variety of conflict mechanisms, including DNA damage, concurrent DNA transcription, and DNA-bound protein complexes which can all act to stall the replication processes. Without a mechanism for rapid and efficient resolution, these conflicts have the potential to cause genomic instability and even cell death. The frequency of these conflicts and their consequences to replisome structure remain unknown. We report the direct visualization of replication conflicts with single molecule sensitivity in the gram-positive bacterium *Bacillus subtilis* and characterize the dynamics of these conflicts *in vivo*.

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RecG Interaction with the DNA Replication Fork. The Role of E. coli SSB Protein

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The RecG DNA helicase is a guardian of the bacterial genome. It binds to a variety of forked DNA structures thereby minimizing pathological DNA